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A REVIEW ON *COLLETOTRICHUM* BLIGHT OF CHICKPEA IN ANDHRA PRADESH

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ABSTRACT

Chickpea is severely affected by Colletotrichum blight caused by Colletotrichum dematium (Persoon) Grove. and Colletotrichum capsici (Sydow) Butler and Bisby. In some of the major chickpea growing areas of Kurnool, Prakasam and Anantapur districts of Andhra Pradesh during rabi 2009 and 2010 due to heavy unusual rains, resulted in failure of the crop in many areas and led to re-sowing of crop in some areas. There is no research on Colletotrichum blight of chickpea in Andhra Pradesh as the disease occurred in severe form in recent years. Hence an attempt was made to study on the disease in which, A total of seven isolates of Colletotrichum blight pathogen were collected from major chickpea growing areas of Kurnool, Anantapur, Prakasam, Kadapa and Nellore districts of Andhra Pradesh. Variability among seven isolates of Colletotrichum capsici causing blight in chickpea with respect to morphological and cultural characteristics was studied. Genetic diversity at molecular level among isolates was studied using molecular technique like RAPD. Management of pathogen by fungicides and bio-control agents was also studied.

KEYWORDS: Colletotrichum dematium (Persoon), Colletotrichum capsici (Sydow)

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INTRODUCTION

Chickpea is a cool season legume crop and is grown in several countries worldwide as a food source. Seed is the main edible part of the plant and is a rich source of protein, carbohydrates and minerals especially for the vegetarian population. As in case of other legume crops, even chickpea can fix atmospheric nitrogen through its symbiotic association with *Rhizobium* sp.; thus helping in enhancing the soil quality for subsequent cereal crop cultivation. Pulses contain two to three times more protein than cereals, ranging from 20 to 40 per cent (Arora, 1988). Hence, the crops have been named as “poor man’s meat and rich man’s vegetable”.

Among the pulses, chickpea (*Cicer arietinum* L.) is the third most important pulse crop of the world, next to field bean (*Phaseolus vulgaris*) and pea (*Pisum sativum*). It is cultivated mainly in semi arid and warm temperate regions of the world especially in *rabi* season.

About 65 per cent of global area with 68 per cent of global production of chickpea is contributed by India (Suhasini *et al.*, 2009). India ranked first in terms of chickpea production and consumption in the world. In India chickpea contributes nearly 42 to 47 per cent of the total pulse production.

Chickpea is the most important pulse crop of India in terms of both area and production. India is the largest producer of chickpea in the world sharing 65.25 and 65.49 per cent of the total area (11.97 m ha) and production

(10.89 mt), respectively. In India chickpea is grown in an area of 8.95 million ha with an annual production of 7.70 million tonnes and 928 kg ha^{-1} productivity (www.indiastat.com). Madhya Pradesh, Uttar Pradesh, Rajasthan, Maharashtra, Gujarat, Andhra Pradesh and Karnataka are the major chickpea producing states sharing over 95 % area. In Andhra Pradesh, it is grown in an area of 5.34 lakh ha with an annual production of 5.20 lakh tonnes and productivity of 920 kg ha^{-1} (www.indiastat.com). Four major chickpea growing districts in Andhra Pradesh are Kurnool (2,40,000 ha), Prakasam (1,00,000), Anantapur (70,000 ha) and Kadapa (70,000 ha) which accounts for nearly 70 to 75 per cent of chickpea area in Andhra Pradesh. Major constraints for potential chickpea yield are diseases, insect pests and poor management practices. Chickpea is affected by 67 fungi, 3 bacteria, 22 viruses, mycoplasma and 80 nematodes (Nene *et al*., 1996). In the order of global importance, the major diseases are *Ascochyta* blight, *Fusarium* wilt, *Botrytis* grey mould, dry root rot, stem rot, foot rot, black root rot, *Verticillium* wilt and rust. In India major diseases of chickpea are *Ascochyta* blight, *Fusarium* wilt, dry root rot and *Botrytis* grey mould. Among these *Ascochyta* blight is a major disease especially in northern parts of India. Dry root rot and *Fusarium* wilt are the major diseases of chickpea prevailing in Andhra Pradesh. In addition to these two diseases, incidence of *Colletotrichum* blight was observed in severe form in Kurnool, Prakasam and Anantapur districts of Andhra Pradesh during *rabi* 2009 and 2010 due to heavy unusual rains which resulted in failure of the crop in many areas and led to re-sowing of crop in some areas.

The studies on symptoms and pathogen proved it as *Colletotrichum* blight caused by *C. capsici* (Uday Krishna, 2012). *Colletotrichum* blight was reported to be caused by *Colletotrichum dematium* (Persoon) Grove and *Colletotrichum capsici* (Sydow) Butler and Bisby. *Colletotrichum* blight is seed and soil borne disease. It is fatal in the early sown September crop when the temperatures are high (25-30°C) and it does not normally occur in post-rainy-season crop, except under unusual rains (Nene *et al*., 1996). Mishra *et al*. (1975) reported this disease in India for the first time from Jabalpur and Indore, Madhya Pradesh during his survey on diseases of pulse crops. They described symptoms of *C. dematium* on chickpea plants as brown to dark brown discoloured sunken lesions at collar region near soil surface. Severely affected plants exhibited yellowing symptoms with poor vigour and died prematurely. Nene *et al.* (1991) also described that the *Colletotrichum* blight shows symptoms on all aerial parts of chickpea. Small water soaked yellowish spots appear on the lower leaves which later turns into circular brown lesions with yellow margins of 1 to 3 mm in diameter. In some cases, lesions enlarge rapidly, become irregular and cover the entire leaflet, and extend to the stems. Lesions on pods are circular to elongate, and sunken at the centre. Plants wilt and dry due to severe infection.

Geographical Distribution and Economic Importance

Colletotrichum is one of the most important genera of plant pathogenic fungi world wide, especially in sub tropical and tropical regions. Washthi and Bhargava (2000) studied yield losses of chickpea due to *Colletotrichum dematium* and reported that diseased plants had significant reduction in pods, plant yield, 100 seed weight and sound seeds.

Host Range

Colletotrichum capsici has been reported to infect many commercially grown crops as well as other plants. A leaf spot disease of turmeric caused by *C. capsici* has been reported from kadapa, Kurnool, Guntur, Krishna and Godavari districts of Andhra Pradesh (Ramakrishnan, 1954). Kothari and Bhatnagar (1966) observed severe infection of cluster bean by *C. capsici* resulting in shortage of fodder in Rajasthan. The fungus *C. capsici* has been reported to infect chilli, bell pepper, aristolochia, bengalgram, cotton, brinjal and many other plants from a wide range of families (Mordue, 1971). A severe damage of brinjal fruits by a fruit rotting fungus, *C. capsici* was observed in the experimental plots of Punjab Agricultural

University (Singh *et al.*, 1973). Boll rot of cotton due to *C. capsici* has been reported from Punjab (Chopra *et al.*, 1974). The fungus has been reported to infect as many as 176 host genera (Sutton, 1980). Sherf and Mac Nab (1986) reported infection of marigold by *C. capsici*. Leaf spot and stem anthracnose caused by *C. capsici* has been found to be a major constraint in betelvine production in West Bengal and other states of India (Gupta and Sen, 1988). Anthracnose of castor caused *by C. capsici* was reported for the first time in India by Sinha and Singh (1993). Pring *et al*. (1995) in their extensive studies on host range found that *C. capsici* (Syd.) Butler and Bibsy can infect *Cicer arietinum, Lupis angustifolius, Phaseolus vulgaris, Pisum sativum, Vigna radiata* and *V. unguiculata.* Field isolates of *C. capsici* obtained from soybean plants could infect bell pepper, tomato, egg plant and cotton seedlings as well as bolls (Roy, 1996). The pathogenicity of isolates was proved on these host plants. Urdbean anthracnose caused by *C. capsici* (Syd.) Butler and Bibsy has been reported from all regions of India in mild to severe form. It causes considerable damage by reducing seed quality and yield (Joshi and Tripathi, 2002). *C. capsici* has been found to cause blight disease in bitter gourd (Samita and Dubey, 2003).

Morphology of the Pathogen

West (1961) opined that small round sclerotia are an outstanding morphological characteristic of the organism and the fungus belongs to the group of non spore producing fungi. In pure culture, initially the fungal mycelium is silky white but gradually losses its luster and becomes somewhat dull in appearance. The mycelium is radiating with abundant aerial hyphae which grow very fast. Often the aerial hyphae appear as dense tufts dispersed all over the culture medium. The mycelium completely disappears over a period of three months leaving only sclerotial bodies. Colonies are very fast growing reaching 9 cm diameter in three days. Sclerotia are white at first and gradually become light brown to dark brown at maturity.

Symptomatology

The disease starts with pre-emergence rot of seeds characterized by rotten and softened seeds which are covered by white profuse, mycelial growth of the fungus. The pathogen attacks the germinated seedlings and causes wilt. The pathogen attacks all the parts of the plant, but the stem infection is the most common and destructive. Yellowing and wilting of branches near the base is the first symptom. Sheaths of white mycelium develop at or near the soil line around the affected areas of the stem. Abundant sclerotia, initially white and later turning brown, develop on the infected areas. The entire plant can be killed, but in some cases two or three branches are affected. Infection of pegs, pods and roots occurs either independently or together with stem infection. Lesions on the developing pegs may retard pod development. Orange or brown coloured lesions may be found on the pods. Severely infected pods are completely covered with a white mycelial mat and eventually decay. In some cases the seeds from the diseased pods show a characteristic bluish grey discolouration of the testa known as blue damage (Subramanyam, 1964). Asghari and Mayee (1991) reported a variety of symptoms in groundnut viz., seed rot, seedling blight, collar and stem rot, peg and pod rot. This disease is of considerable economic significance for groundnut grown under irrigation, particularly in post rainy season. Seed rot and blight are less common than stem and pod rot damage. Varaprasad (2000) described the symptoms of chickpea blight caused by *C. dematium* in Dharwad, Karnataka. The infected plant showed brown to dark brown discoloured sunken lesions at collar region near the soil surface. The necrotic lesions extended upto 5 to 6 cm from soil surface. The infection spread to the central branch which was completely girdled by the blight. In the severely affected plants yellowing of lower leaves become conspicuous and gave burnt appearance of the plants.

The Causal Organism

Ramakrishnan (1947) reported that *Colletotrichum* sp. observed on chickpea was considered to be *C. capsici*. *Colletotrichum* blight is caused by *Colletotrichum dematium* and *Alternaria* blight is caused by *Alternaria alternata* as reported by Vishwakarma and Chandam (1974). Mishra *et al.* (1975) first time reported chickpea blight caused by *C. dematium* on Kabuli variety in India from Jabalpur and Indore, Madhya Pradesh during the survey on diseases of pulse crops. Sutton (1980) had given a key and description to the 22 species recognized in this genus based on cultural characters. The variability among *Colletotrichum* species have been described by various authors (Sutton, 1980; Sutton, 1992). Currently around forty species are accepted based on more detailed studies on morphology, cultural characters and pathogenic abilities (Cannon *et al*., 2000). Among *Colletotrichum* species, *Colletotrichum capsici* causes blight on chickpea and other diseases in chillies, turmeric etc. Nene *et al*. (1996) reported *Colletotrichum* blight of chickpea was caused by *Colletotrichum dematium* (Persoon) Grove. and *Colletotrichum capsici* (Sydow) Butler and Bisby. Weeraratne and Chithral (1997) screened 240 varieties from ICRISAT and 423 lines from ICARDA and presented information on varietal resistance to wilt (*Fusarium* sp.), *Colletotrichum* blight (*C. dematium*) and *Alternaria* blight (*A . alternata*). An intensive roving survey on *Colletotrichum* blight was conducted by Varaprasad (2000) during *rabi* 1998-99 in and around Gulbarga, Jewargi, Afzulpur, Sedam and Aland talukas of Karnataka state where chickpea is predominantly grown and reported that the disease was more prevalent in low lying areas and maximum disease incidence of 67.84 per cent was recorded in Gulbarga taluk ranged from 0 to 91 per cent.

Colletotrichum blight pathogen from infected chickpea plants of Nandyal region, Kurnool district, Andhra Pradesh was isolated and identified as *C. capsici* (Uday Krsishna, 2012). Anthracnose fungus has been described by a number of workers (Mordue, 1971; Sutton, 1980; Roberts and Snow, 1984; Akinwunmi and Latunde, 2001). The imperfect stage of the fungus, *Colletotrichum capsici* (Syd.) Butler and Bibsy causes anthracnose of chilli, cotton, urdbean and many leguminous hosts as well as vegetable and perennial fruit crops. The name *C. capsici* was created by Butler and Bibsy (1931) to include most forms of *Colletotrichum* with falcate conidia. Sutton (1980) distinguished *Colletotrichum capsici* by the wider conidia and Mordue (1971) by its pathogenicity. The type species was placed in *Vermicularia* by Sydow in 1913 and later as *Colletotrichum* by Butler and Bibsy (1931).

Taxonomy of the Pathogen

Members of the genus *Colletotrichum* belong to the sub-division Deuteromycotina, the imperfect fungi reproduced by mitotically produced spores. The sub-division has been further divided into two classes, whereby *Colletotrichum* has been systematized to belong to class Coelomycetes (conidia generated in pycnidial, pycnothyral, acervular, cupulate or stromatic conidiomata) and further as a member of the order Melanconiales (conidia produced in acervuli) (Hawksworth *et al*., 1983). As the perfect stage for *Colletotrichum* is not commonly found in nature or has not been described for certain species, most taxonomic approaches to establish relationship within these groups have relied on the morphology of the asexual stage. As mentioned by Sutton (1980), several hundred species of *Colletotrichum* were recognized at the beginning of the twentieth centuary. Von Arx (1957) reduced and reclassified the genus into 11 species. Sutton (1980) suggested 22 species of *Colletotrichum* should be differentiated. Due to aspects involving host specificity, this number has been increased to 39 species including same species with physiological taxa (Sutton, 1992). *C. capsici* was first described as *Vermicularia capsici* in 1913 by Sydow from pepper fruit collected by Mc Rae in India in 1912. Ramakrishnan (1954) considered *C. capsici* and *C. indicum* as synonyms. Both species epithets have since been applied to falcate spored

Colletotrichum species. Von Arx (1957) placed *C. capsici*, *C. indicum* and numerous other graminicolus falcate spored species in synonyms with *C. dematium*. Mordue (1971) considered *C. dematium* to be a temperate non pathogenic species with narrow conidia and *C. capsici* to be a tropical and sub-tropical pathogen with some what broader conidia. Sutton (1980) has recognized several distinct species in the *C. dematium* complex proposed by Von Arx (1957) and *C. capsici* is one such taxa. However molecular biology provides new insight into systematics, particularly in delimitation of species and defining inter and intra specific relationship (Sharma *et al*., 2005).

Isolation of the Pathogen and Pathogenicity Tests

Colletotrichum capsici which causes leaf spot disease of turmeric (*Curcuma longa* L.) could also infect chickpea on artificial inoculation as observed by Ramakrishnan (1954). Rai and Chohan (1966) conducted pathogenicity test on chilli plant bearing 20 fruits by spraying with spore suspension of different isolates of *C. capsici*. The inoculated plants were placed in humid chamber which were kept moistened throughout the period of test. Observations on the per cent fruit rot caused by different isolates, number of days taken to produce symptoms, virulence and intensity of spotting on the leaves were recorded. Isolates A, B and D were found to be highly virulent and expressed the symptoms within 15 days. Kenchaiah (1975) proved the pathogenicity of two isolates of *C. capsici* using both ripe and unripe fruits of *Capsicum annum* and *Capsicum frutescens* and reported that they were pathogenic to the respective hosts and were cross incurable. Singh *et al.* (1977) isolated *Colletotrichum capsici* from a severely infected chilli fruit and proved pathogenicity experimentally. Cardwell *et al*. (1989) reported the existence of eight different pathotypes among the twelve isolates of *C. graminicola* based on their virulence pattern on a set of eight sorghum differential lines. Roberts and Snow (1990) carried out morphological and pathological studies of *C. capsici* and *C. indicum* for evaluating synonymy in these closely related taxa. The four isolates of *C. capsici* recovered from pepper and cotton could infect cotton bolls but the number of bolls infected varied with the isolate. Jayalakshmi *et al*. (1998) indicated the presence of physiologically different types of isolates of the pathogen, *C. capsici* infecting chilli. Of the five isolates tested for virulence, one was found most virulent. Varaprasad (2000) carried out artificial inoculation of *C. dematium* on 2 to 3 week old chickpea seedlings and reported that the symptoms appeared as water soaked irregular brown discolouration on basal region of the stem and also on branches, six days after inoculation. Srinivasan Madhavan *et al.* (2010) reported that *Colletotrichum capsici* was the most commonly isolated fungal species than *Colletotrichum gloeosporiodes* and *Alternaria alternata* from infected chilli fruits, whereas the measurement of disease severity of chilli revealed differences in the virulence between isolates. Uday Krishna (2012) isolated *Colletotrichum capsici* from severely infected chickpea plants and proved pathogenicity experimentally.

Morphological and Cultural Characteristics of the Isolates

The mycelium of *C. capsici* is immersed, branched, septate, hyaline light to dark grey in colour. It is characterized by conidiomata called acervulus which is present in the necrotic part of the lesion (Sutton, 1980). Acervuli on leaf, stem and fruit appears black rounded or elongated approximately 350 µm in diameter. Occasional cells of the acervulus develop as setae which are 1-5 septate, brown, slightly swollen at the base, then tapered tip. Conidiophores were numerous, unicellular, hyaline to faintly brown, cylindrical on which conidia are formed singly (Mordue, 1971).

According to CMI descriptions the conidial length of isolates fell within the range of 16-30 µm. Khirbat *et al*. (1980) while studying physiological and morphological variability in the sugarcane red rot fungus, *C. falcatum* observed differences in colony growth and sporulation among the five different isolates collected from different localities in Haryana. Gupta (1981) studied the variations in *C. capsici* isolates (Col. 1-9 and Col. 12-14) from different locations in

West Bengal. Isolates from different localities differed in their acervuli production, sporulation and virulence in causing anthracnose disease on betelvine plants. Among the twelve isolates tested, the intensity of acervuli production was highest in isolate Col. 4 and least in Col. 9 whereas the acervuli production was high in isolates Col. 3, 5 and 14. Intensity of sporulation per acervulus was perceptibly higher in isolates Col. 4, 13 and 14 and the lowest was in Col. 5. Sporulation was highest in Col. 4 and least in isolates Cols. 2, 3, 5, 6 and 9. The size of conidia, measurements of setae showed variation among the twelve isolates of *C. capsici* causing anthracnose of betelvine in West Bengal (Gupta, 1988). Thind and Jhooty (1990) studied variation in colony diameter, colony growth, growth rate and the pattern of acervulus formation among seven isolates of *C. capsici* causing fruit rot of chilli in Punjab.

Colony diameter ranged from 52.13 to 90 mm and the growth rate ranged from 4.3 to 7.5 mm per day. Acervuli have shown either scattered or in the form of concentric rings. Angadi (1999) found that among the solid media, potato dextrose agar was best for the growth of *C. capsici* on chilli crop, the maximum mycelial weight was observed after 16 days of incubation. Among the various liquid media used, maximum dry mycelial weight was observed in Richard's medium. Four chilli isolates of *C. capsici* were observed for the difference in size and shape of conidia, shape of setae, number of setae per acervulus, number of septa per setae and sporulation. All the four isolates produced short, hyaline, conidiophores bearing hyaline, falcate conidia singly with a centrally placed oil globule. But one isolate designated as I_1 produced maximum number of setae per acervulus with more number of septa (Jeyalakshmi and Seetharaman, 1999). Varaprasad (2000) reported that Sabouraud's and Richard's agar supported good growth of *C. dematium* on chickpea. Similarly, out of 10 liquid media tested, Richard's medium supported good mycelial growth and sporulation. Shinde *et al*. (2003) reported that abundant germination and growth of spore occurred at 30°C followed by 25°C on PDA. Poor growth was observed at 40°C and no growth at 45°C. Better growth and development of acervuli took place under the alternate cycle of light and darkness. A mean colony diameter of 70.50 mm (on day 7) and abundant acervuli formation were observed in alternate cycle of light and darkness compared to continuous light and continuous darkness (65 and 42 mm diameter, respectively).

Poor growth and meager acervuli developed under complete darkness, indicating that light is essential for growth and sporulation. Spore diameter was highest and spore germination was abundant on PDA, followed by Czapek's and Richard's agar media. Sinha *et al.* (2004) conducted laboratory experiments to determine the effect of pH, temperature and polythene colour on the growth, sporulation and spore germination of *C. capsici* and reported that the maximum radial growth and sporulation was observed at pH 7.0, 30°C temperature and in blue coloured polythene while minimum at pH 4.0, 15°C temperature and in green coloured polythene. Gorawar *et al.* (2006) studied turmeric leaf spot disease and observed that the acervuli *of C. capsici* were hemispherical in shape and conidia are single celled hyaline, smooth walled, falcate or sickle shaped with blunt ends measuring 16-24 × 2.5-3.5 µm and reported growth parameters of *C. capsici*, cultured in different culture media and concluded that among the solid media, malt extract and host leaf extract agar showed highest radial growth of *C. capsici* (90 mm), followed by potato dextrose (85.0 mm) and maize meal (81.70 mm) agar medium. Toyozo Sato *et al.* (2008) reported that fungal isolates of *Colletotrichum capsici* formed white to greyish colonies on PDA at 27°C in the dark.

Acervuli and pale salmon colored conidial masses were developed on PDA at 20°C under the near ultra violet irradiation. Seta were dark brown, 1~4-septate, 78~210 × 3.5~6.5 µm in size. Conidia were hyaline, one-celled, smooth, guttulate, falcate, 16~26 × 2.4~4 µm (av. 22.3 × 3.2 µm) in size. Appressoria were sepia brown, ovoid to irregular,

8~16(~20) × 5~11 µm in size, often born on tips of chained chlamydospore-like cells. Vinaya Hemannavar (2008) observed the cultural and morphological characters of *C. capsici* causing chilli anthracnose and reported that the colonies were light brownish black having more whitish aerial mycelial growth. Margins were smooth with white cottony growth. Characteristic fine concentric ring of growth had seen against light. Acervuli are gregarious, abundant, sub conical when young, circular to saucer shaped at first covered by host tissue, then erumpent and blackish covered by stiff divergent setae. Diameter of acervuli ranges between 53.3 to 136.4 µm in host and 71.0 to 161.9 µm in culture. Setae are dark in colour but paler at the apex, swollen at the base and tapering at the apex and more or less erect and upto 150 µ long. Conidia are one celled, hyaline, smooth walled, with a central oil globule, curved, sickle shaped, tapering gradually at both ends with acute apex and measured 16-30 × 2.5-4 µ in size. Wasantha Kumara and Rawal (2008) identified the response of different isolates to different temperature levels were found to be vary and most of the isolates preferred temperature range of 28°C to 30°C for the growth and sporulation when grown on Richard's agar medium and also reported that isolates of *Colletotrichum gloeosporioides* grew well at pH 5 while sporulation was better at pH 6. Lubna Masoodi *et al*. (2012) studied 20 isolates of *C. capsici* and reported that the colour of colonies of *C. capsici* ranged between white to grey. Growth rate of isolates was between 32.0-67.5 mm. Morphological studies of isolates revealed variations in their colony colour, acervuli production, size and shape of setae and conidia. Average conidial length and width varied from 33.6 µm and 2.23 µm respectively and average length and width of setae varied from 177.21 µm and 4.48 respectively.

Molecular Variability among the Isolates

Random Amplified Polymorphic DNA (RAPD) markers provide a mechanism for swiftly and easily characterizing differences between isolates in terms of polymorphism of primer defined DNA fragments. Each band amplified by the use of random primer would represent a specific locus in the genome, and alleles would produce easily identified band of a different size. Arbitrarily primed-PCR (ap-PCR) or Random Amplified Polymorphic DNA (RAPD) has been extensively used for identification and characterization of isolates in *Colletotrichum* (Sreenivasaprasad *et al*., 1992; Sreenivasaprasad *et al*., 1993; Alahakoon *et al*., 1994). Bean anthracnose fungus, *Colletotrichum lindemuthianum* exhibits diverse physiological races even in the absence of asexual stage. RAPD analysis was used to assess genetic basis of such variability. A selection of twelve isolates originating from diverse localities was choosen and analysed for molecular variability. Two separate molecular groups were identified based on DNA polymorphism (Fabre *et al*., 1995). Random amplified polymorphic DNA profiles were used to know the extent of genetic relatedness in *Colletotrichum graminicola* isolates from turf grass and annual blue grass with that of isolates from corn and sorghum (Browning *et al*., 1999). Fifty seven per cent genetic similarity was found between the isolates from turf grass and isolates from corn and sorghum. Within the turf grass isolates six distinct clusters were observed.

Pattern of bands on the electrophoretic gel indicated DNA polymorphism among the twelve isolates of *Colletotrichum graminicola*, which varied in length of the fragment amplified and number of fragments. Similarity index values revealed 42.5 per cent polymorphism in the banding pattern indicating genetic variability (Hazra *et al*., 1999). Crown rot pathogen isolates of strawberry *C. gloeosporiodes* were recovered from strawberry as well as adjacent non-cultivated hosts and subjected to RAPD analysis to determine the extent of genetic diversity in them (Xiao *et al*., 2004). All the isolates from both the sources were separated into two clusters based on the banding pattern indicating the non-cultivated hosts as potential sources of inoculums. Molecular analysis based on sequences of the rDNA Internal Transcribed Spacers (ITS1 and ITS2) among thirty four isolates of *Colletotrichum* spp. from banana, ginger, *Euphatorium*

thymifola, soybean, longan, mango and *Draceana sanderiana* indicates that the *Colletotrichum* isolates comprised four clades that paralled the morphological groupings.

Most isolates clustered within three distinct clades which potentially represented different species. The correlation between morphological and molecular based clustering demonstrated the genetic relationships among the isolates and species of *Colletotrichum* and indicated that ITS rDNA sequence data were potentially useful in taxonomic species determination (Wipornpan photita *et al*., 2005). RAPD analysis was performed on eighteen isolates including two species, *Colletotrichum gloeosporioides* and *C. capsici* and relationship among the species were analyzed based on the dendrogram of RAPD patterns using UPGMA (Unweighed Pair Group Method with Arithmetic Mean) and RAPD analysis showed a clear difference between *Colletotrichum gloeosporioides* and *C. capsici* and furthermore *C. capsici* were more closely related than *C. gloeosporioides* isolates (Ratanacherdchai *et al*., 2007). Ten isolates of *Colletotrichum capsici* causing chilli anthracnose were collected from ten provinces in the northeast of Thailand and molecular polymorphism was generated among the ten isolates by RAPD confirmed the variation of the different isolates and they were grouped into two clusters (Aphidech Sangdee *et al*., 2011).

Management of Pathogen by Fungicides and Bio-Control Agents

Efficacy of Fungicides against *Colletotrichum Capsici*

Mesta (1996) reported that among the non-systemic fungicides mancozeb, captan and chlorothalonil were found to be highly effective in inhibiting the growth of *C. capsici* at 3000 ppm concentration and among the systemic fungicides carbendazim, bitertanol and tridemefon were found effective at 1000 ppm concentration. Among the non-systemic fungicides mancozeb (DM-45) was found to be highly effective in inhibiting growth and germination of conidia of *C. capsici* at 3000 ppm, whereas among systemic fungicides carbendazim was found effective at 1500 ppm (Hegde, 1998). While studying *in vitro* evaluation of fungicides against *C. dematium* in chickpea, Varaprasad (2000) found that carbendazim (0.1%), kitazin (0.3%) were found effective among systematic fungicide in inhibiting the growth of fungus, while mancozeb (0.2 and 0.3%) was found to be superior among the non-systemic fungicides. Hegde *et al.* (2002) tested the efficacy of three triazole fungicides viz., hexaconazole (0.1%), propiconazole (0.1%) and triadimefon (0.1%) against chillies fruit rot pathogen *C. capsici* by poisoned food technique. Significant inhibition of mycelial growth was recorded with all 3 fungicides.

Madhusudhan (2002) tested ten fungicides *in vitro* against *C. truncatum* and reported that benomyl, carbendazim and prochloraz were found superior among systemic fungicides in inhibiting the growth of the fungus. Benomyl and carbendazim inhibited 100 per cent mycelial growth of the fungus at all the three concentrations tested (0.025, 0.05 and 0.1%). SAAF was found to be superior among the non-systemic fungicides by inhibiting 99.22 and 85.92 per cent at 0.25 and 0.20 per cent concentrations, respectively. Chander Mohan and Prem Raj (2004) used different fungicides for control of chilli fruit rot (*Colletotrichum capsici*) and Stemphylium blight (*Stemphylium botryosum*) of onion and reported that triazole fungicides exhibited the highest pathogen inhibition. Of the three triazoles, tebuconazole (folicur) was the most effective with ED50 values of 5.5 and 12.0 micro gml^{-1} for *S. botryosum* and *C. capsici*, respectively, followed by Score and Contaf. The three contact fungicides (Antracol, Indofil M-45 and Kavach) also showed good control of blight. The triazole fungicides produced the highest yields.

Shinde and Raut (2005) reported that mancozeb and thiram recorded 100 per cent growth inhibition of *Colletotrichum dematium* in chickpea and were the most effective fungicides followed by thiophanate methyl (97.41%),

carbendazim (97.13%), captafol (96.37%), zineb (95.86%) and copper oxy chloride (94.82%).Laxman (2006) found that among eight different systemic fungicides tested *in vitro* against *C. truncatum* in green gram, most of them showed complete inhibition of mycelial growth at the concentration of 0.05 and 0.1 per cent except tricyclazole and hexaconazole. However, tricyclazole and hexaconazole were found most effective at 0.15 per cent. Among non-systemic fungicides, wettable sulphur was very effective at all concentrations followed by mancozeb. Shovan *et al.* (2008) evaluated five fungicides *viz.*, propiconazole, carboxin + thiram, iprodione, mancozeb and copper oxychloride at 100, 200 and 400 ppm concentrations for their efficacy against the radial colony growth and mycelial dry weight of *C. dematium* on soybean. The complete inhibition was obtained with propiconazole at all the selected concentrations.

Efficacy of Bio-Control Agents Against *Colletotrichum Capsici*

Trichoderma viride, T. harzianum and *T. koningii* inhibited mycelial growth of *C. capsici* by 51.7, 56.6 and 42.5 per cent respectively (Jayalakshmi *et al.*, 1998). Varaprasad (2000) tested six biocontrol agents for the control of *C. dematium* causing blight of chickpea. Out of them, *T. koningii* (TNAU) inhibited maximum growth followed by *T. harzianum* (UASD). Among the four biocontrol agents tested *in vitro against C. capsici* causing leaf spot of turmeric, *Pseudomonas fluorescens* was found to be superior in inhibiting the growth of the fungus followed by *T. harzianum and T. viride* (Chidanandaswamy, 2001). D'Souza *et al.* (2001) screened eight isolates of *T. harzianum* against *C. capsici* and observed that isolates T1, T2 and T3 of *T. harzianum* were found promising as the biocontrol agents under *in-vitro* conditions. Pathania *et al.* (2004) reported that *Trichoderma hamatum* was found to be effective biological control agent against *C. capsici* followed by *T. viride*, *Bacillus* sp. and *P. fluorescens.* In the laboratory experiments conducted by Chirame and Padule (2005) evaluated the antagonistic activity of *Trichoderma viride, T. hamatum,* T. *harzianum, T. longiflorum* and *T. koningii* against *Colletotrichum capsici* on chillies by direct bit placement method and reported that all biological control agents significantly inhibited the growth of *C. capsici* and *T. hamatum* was significantly superior to the other antagonists as it recorded per cent inhibition of 94.88. *Trichoderma viride, T. harzianum* and *T. virens* inhibited mycelial growth of *C. capsici* on chillies by 71.5, 56.6 and 42.5 per cent respectively as reported by Mandeep and Sharma (2006). Belge and Padghan (2009) reported that *Trichoderma viride* inhibited 40.89 per cent of growth of *C. dematium.* Ekefan *et al.* (2009) found that the *Trichoderma harzianum* isolates suppressed the growth of *C. capsici* resulting in reduction of inoculum on seeds of pepper. Uday Krishna (2012) concluded that *Trichoderma Koningii* showed highest rate of inhibition compared to *Trichoderma viride* aganist *C. capsici* in chickpea by dual culture technique. Among different bio-control agents tested against leaf spot of turmeric caused by *Colletotrichum capsici, T. harzianum* was found to be the most effective antagonist which caused growth inhibition of 53.33 per cent, which showed the possibility of most eco-friendly and inexpensive method of control of leaf spot disease (Jagtap *et al*., 2013).

CONCLUSIONS

Colletotrichum blight of chickpea occurred in severe form in recent years which led to failure of crop in many areas. Among different isolates collected major chickpea growing areas of Andhra Pradesh a great variability of morphological, cultural and molecular variability exists among isolates. Further more *invitro* evaluation of different fungicides and bio control agents revealed that Tebeconozale and *Trichoderma koningii* repectively were more effective.

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